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(12) # EUROPEAN PATENT APPLICATION

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(54) **A process for the incorporation of foreign DNA into the genome of dicotyledonous plants.**

(57) A process is disclosed for the incorporation of foreign DNA into the genome of dicotyledonous plants comprising infecting these plants or incubating dicotyledonous plant protoplasts with bacteria suitable or made suitable for that purpose, which are provided with one or more tumour-inducing plasmids or derivatives therefrom, originally originating from *Agrobacterium*, or from bacteria which contain the T-DNA originating from the above-meant plasmids, and/or the virulence genes originating from the above-mentioned plasmids, incorporated elsewhere in the bacterial DNA.

CLAIMS

1. A process for the incorporation of foreign DNA into the genome of plants, by infecting these plants or explants from them, or incubating the plant protoplasts or cells with bacteria suitable or made suitable for that purpose, characterized in that dicotyledonous plants are infected or dicotyledonous plant protoplasts are incubated with bacteria suitable or made suitable for that purpose, which are provided with one or more tumour-inducing plasmids or derivatives therefrom, originally originating from Agrobacterium, or from bacteria which contain the T-DNA originating from the above-meant plasmids, and/or the virulence genes originating from the above-mentioned plasmids, incorporated elsewhere in the bacterial DNA.

2. A process according to claim 1, characterized in that for the infection or incubation use is made of Rhizobium bacteria or Phyllobacterium bacteria.

3. A process according to claim 1 or 2, characterized in that bacteria are applied which are provided with one or more Ti- or Ri-plasmids or derivatives therefrom.

4. A process according to claim 3, characterized in that

tne bacteria used have been provided with a stable cointegrate plasmid, constructed from a plasmid R772 and a plasmid pTiB6 with foreign DNA incorporated in the T-region of the latter.

5. A process according to any of the preceding claims, characterized in that bacteria are used, which contain at least one plasmid, which has the Vir-region of a tumour-inducing plasmid but no T-region, and at least one other plasmid, which has a T-region with incorporated therein foreign DNA but no Vir-region.

6. Dicotyledonous plants and plant cells obtained after, applying the process according to any of the preceding claims, the generic properties of the original plants or plant cells have been changed.

7. A process for the preparation of chemical and/or pharmaceutical products, characterized in that cells obtained with application of the process according to any of the claims 1-5 are cultivated and the desirable substance is isolated.

8. A process according to claim 7, characterized in that culturing is effected by means of fermentation and if useful subsequent immobilisation.

9. A process according to any of the claims 1-5 incl. or 8, characterized in that the regulator regions positions before and behind thd protein coding regions of T-DNA genes, in particular the genes for octopine synthesis for expressing foreign genes in dicotyledonous plant

cells are used.

10. Dicotyledonous DNA having a portion artificially inserted in it with the process according to any of the preceding claims.

11. Cell lines and regenarated plants obtained after application of the process according to any of the claims 1-9.

12. Rhizobium trifolii LPR 5087 and mutants thereof.

13. Phyllobacterium LAZ100 and mutants thereof.

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(54) **Anti-sense regulation of gene expression in plant cells.**

(57) Regulation of expression of genes encoded for in plant cell genomes is achieved by integration of a gene under the transcriptional control of a promoter which is functional in the host and in which the transcribed strand of DNA is complementary to the strand of DNA that is transcribed from the endogenous gene(s) one wishes to regulate. The integrated gene, referred to as anti-sense, provides an RNA sequence capable of binding to naturally existing RNAs, exemplified by polygalacturonase, and inhibiting their expression, where the anti-sense sequence may bind to the coding, non-coding, or both, portions of the RNA. The anti-sense construction may be introduced into the plant cells in a variety of ways and be integrated into the plant genome for inducible or constitutive transcription of the anti-sense sequence. A wide variety of plant cell properties may be modified by employing this technique.

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European Patent Office

EUROPEAN SEARCH REPORT

Application Number

EP 87 30 2367

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.4)
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The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	20-01-1989	MADDOX A.D.

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
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Place of search	Date of completion of the search	Examiner
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EUROPEAN SEARCH REPORT

Application Number

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DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.4)
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E	WO-A-8 801 645 (MACQUARIE UNIVERSITY) * Claims *	1-3,6-10	
E	EP-A-0 271 988 (ICI PLC) * Whole document *	1-11	
			TECHNICAL FIELDS SEARCHED (Int. Cl.4)

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
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